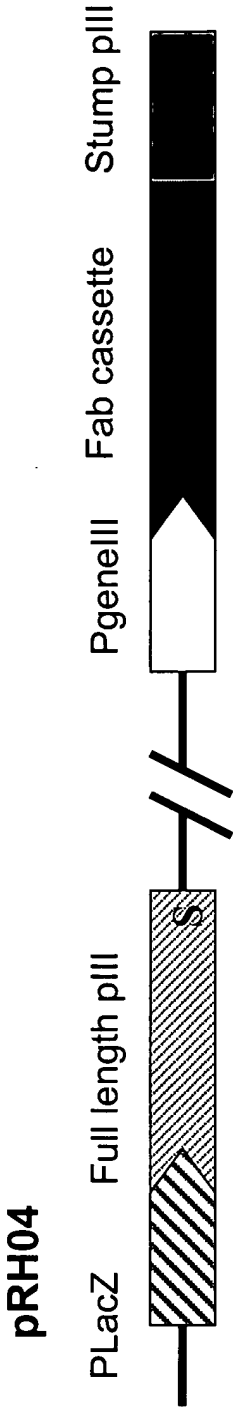
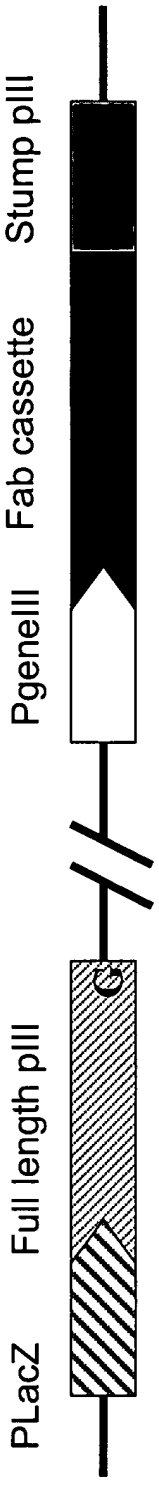

pRH04
PLacZ
Full length pIII
PgeneIII
Fab cassette
Stump pIII

FIG 1A


pRH05
PLacZ
Full length pIII
PgeneIII
Fab cassette
Stump pIII

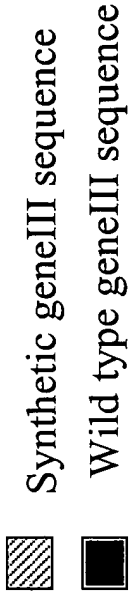

Synthetic geneIII sequence
Wild type geneIII sequence

FIG 1B

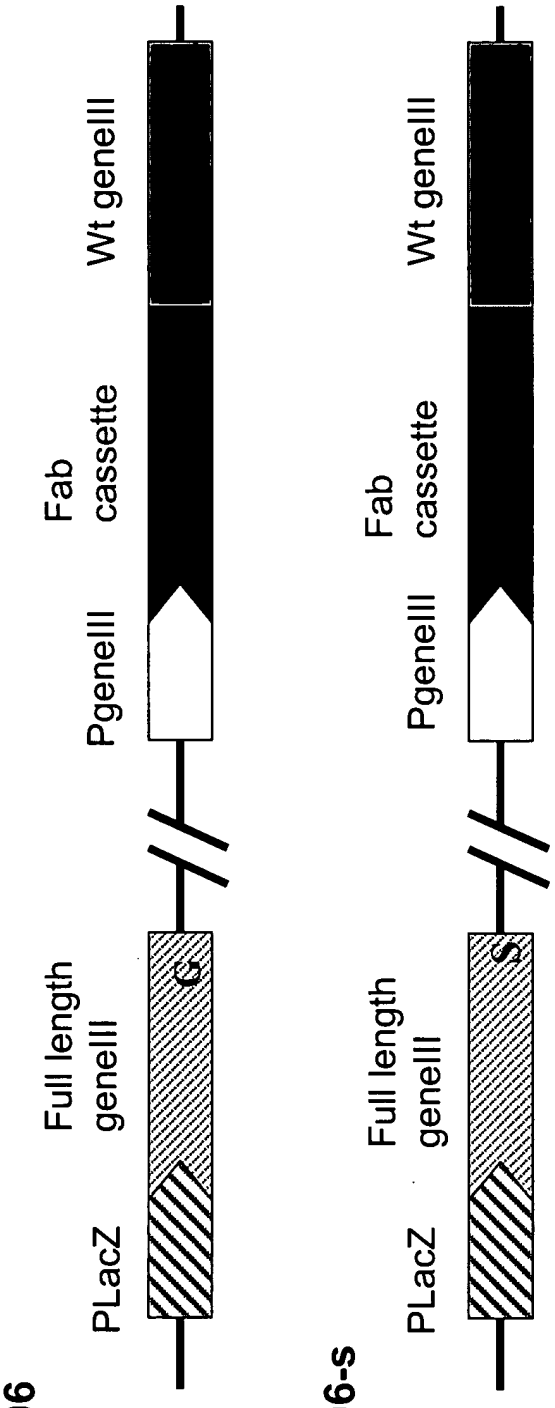

pRH06
PLacZ
Full length geneIII
G
PgeneIII
Fab cassette
Wt geneIII
pRH06-s
PLacZ
Full length geneIII
S
PgeneIII
Fab cassette
Wt geneIII

FIG 1C

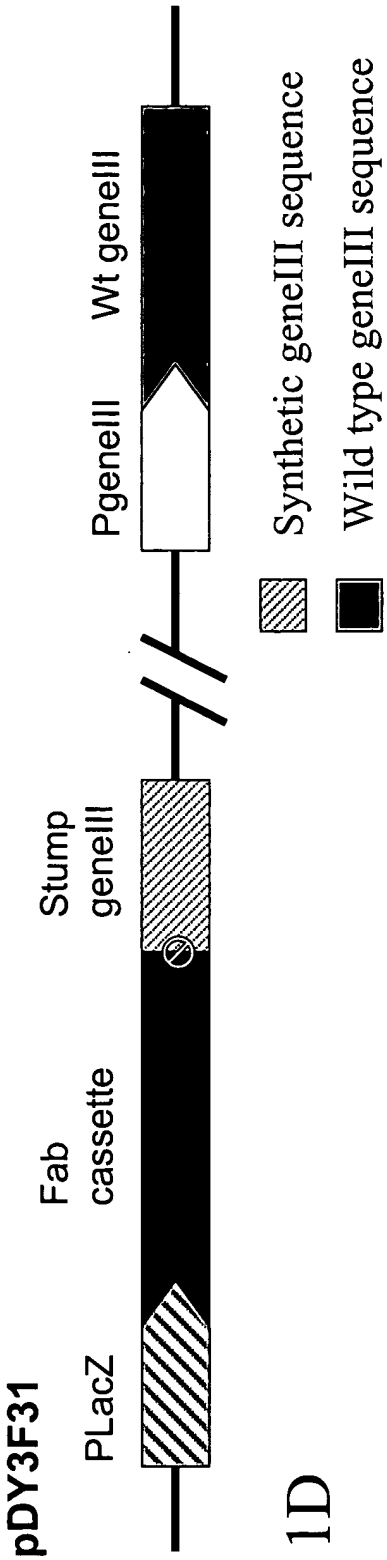

pDY3F31
PLacZ
Fab cassette
Stump geneIII
PgeneIII
Wt geneIII
Synthetic geneIII sequence
Wild type geneIII sequence

FIG 1D

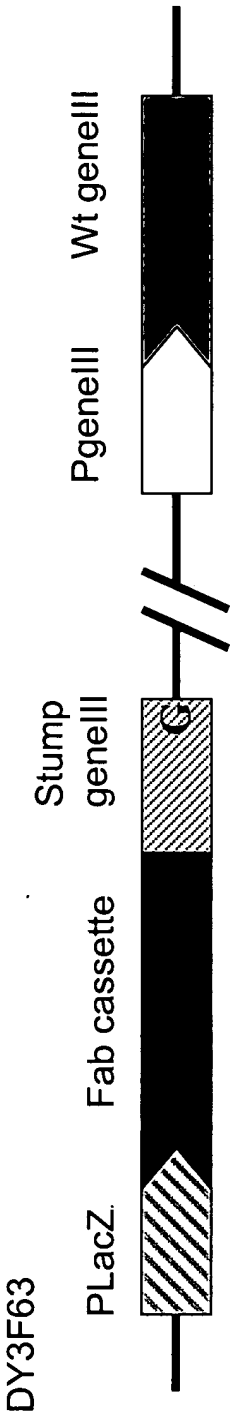


FIG 1E

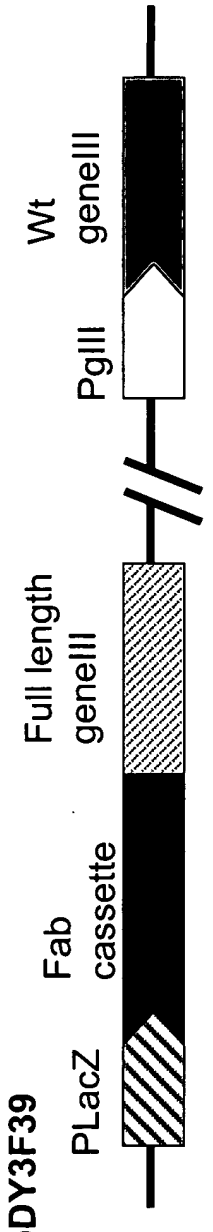
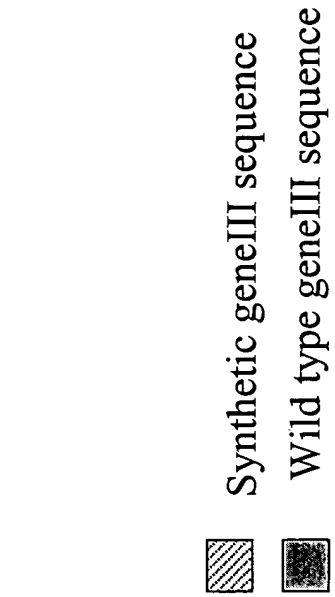


FIG 1F



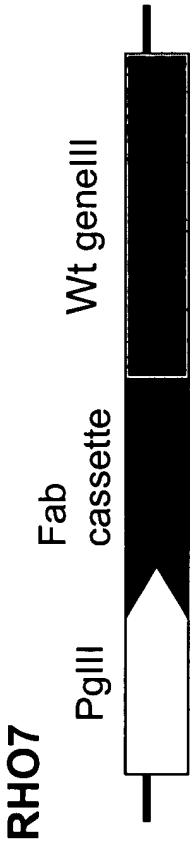
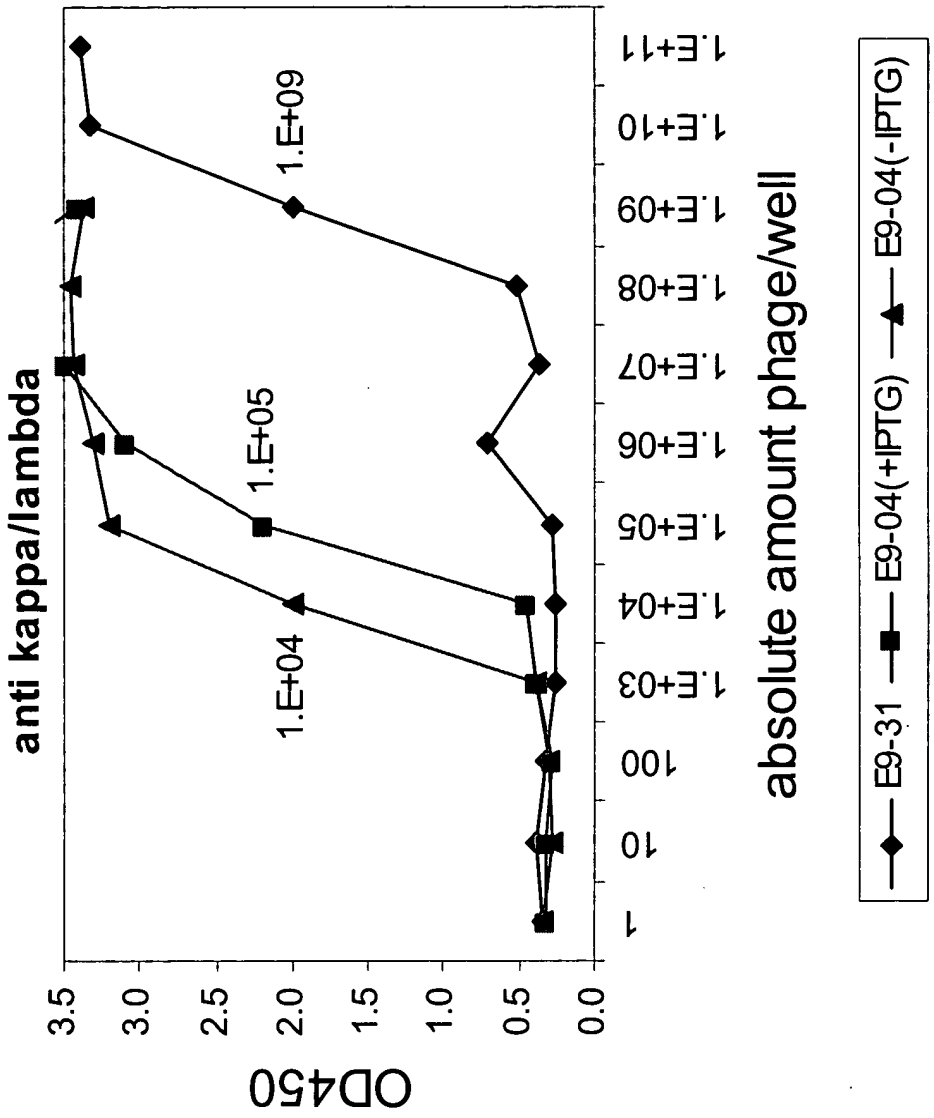


FIG 1G

FIG 2


anti kappa/lambda
OD450
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.E+04
1.E+05
1.E+09
1
10
100
1.E+03
1.E+04
1.E+05
1.E+06
1.E+07
1.E+08
1.E+09
1.E+10
1.E+11
absolute amount phage/well
E9-31
E9-04(+IPTG)
E9-04(-IPTG)

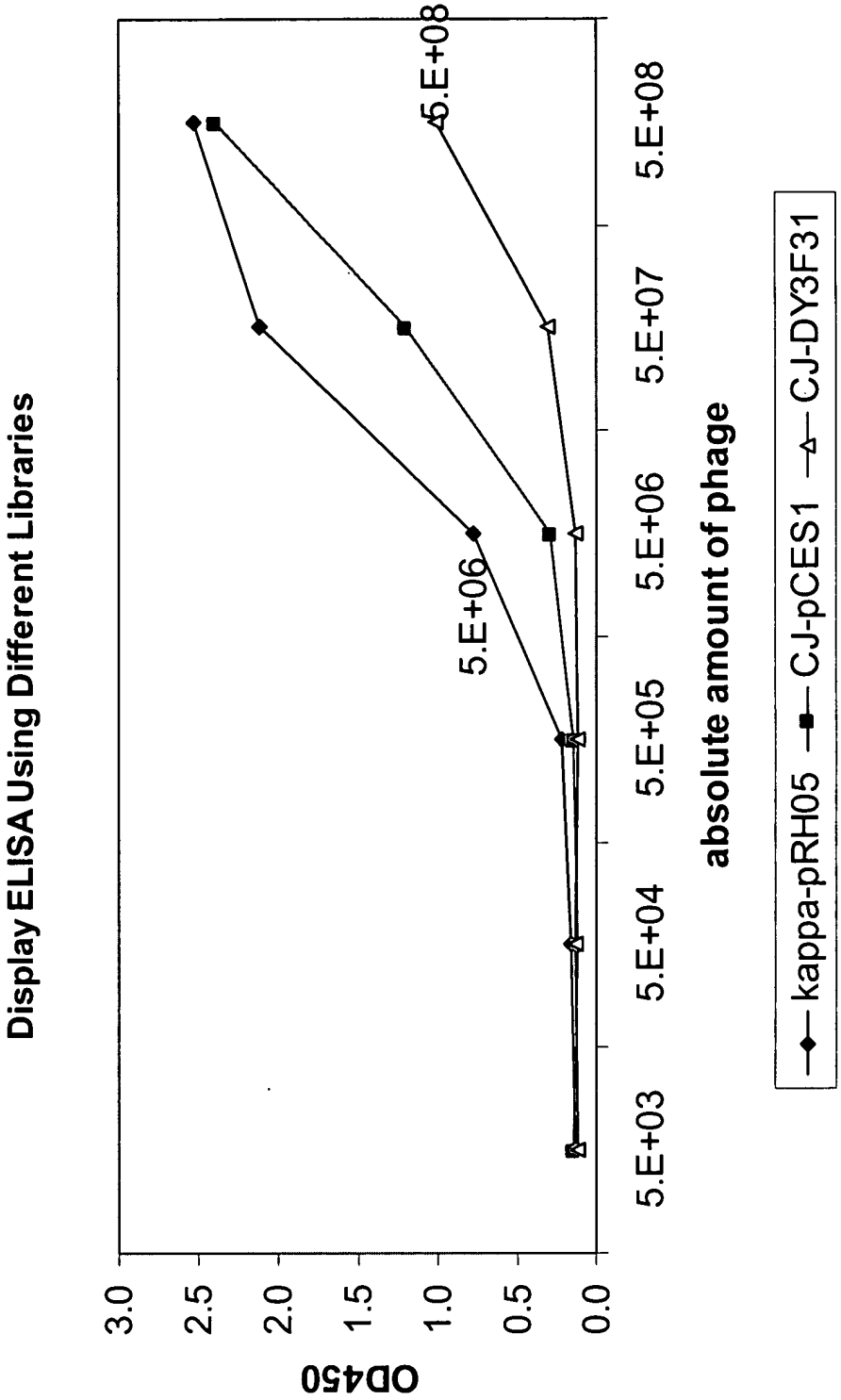

Display ELISA Using Different Libraries
OD450
3.0
2.5
2.0
1.5
1.0
0.5
0.0
5.E+03
5.E+04
5.E+05
5.E+06
5.E+07
5.E+08
absolute amount of phage
5.E+06
5.E+08
kappa-pRH05
CJ-pCES1
CJ-DY3F31

FIG 3

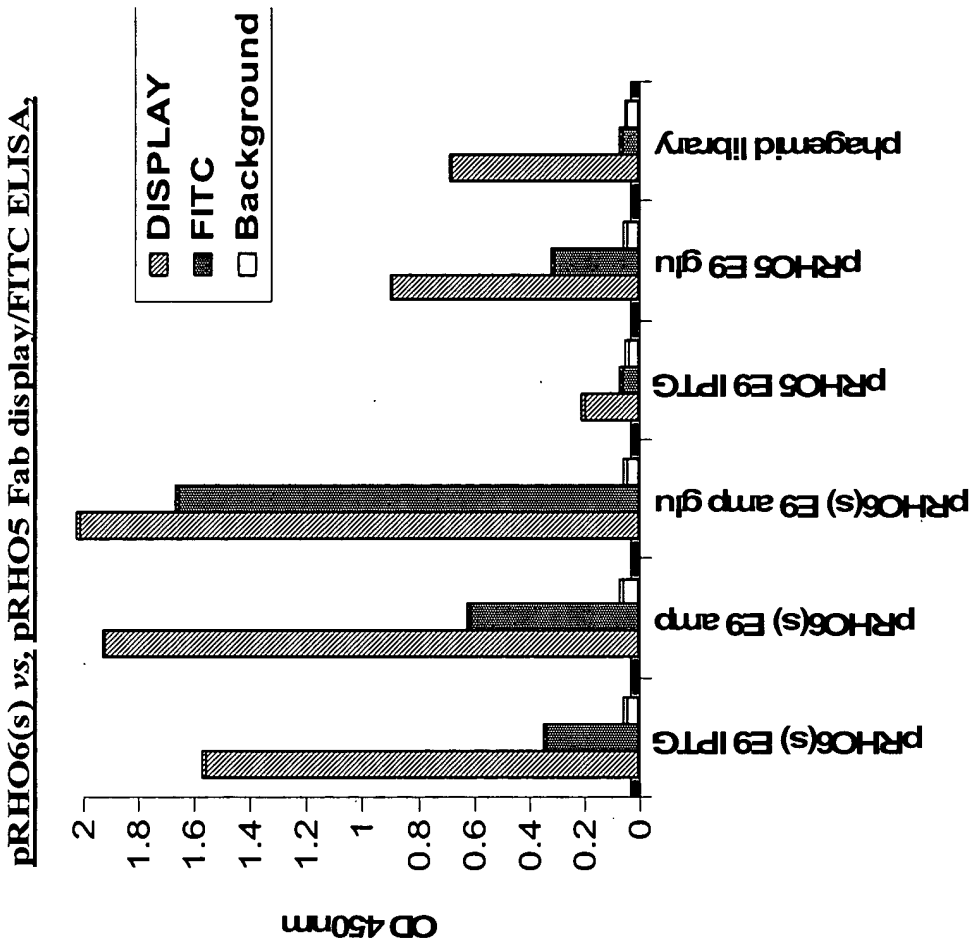

pRHO6(s) vs. pRHO5 Fab display/FITC ELISA;
DISPLAY
FITC
Background
OD 450nm
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
pRHO6(s) E9 IPTG
pRHO6(s) E9 amp
pRHO6(s) E9 amp glu
pRHO5 E9 IPTG
pRHO5 E9 glu
phagemid library

FIG. 4

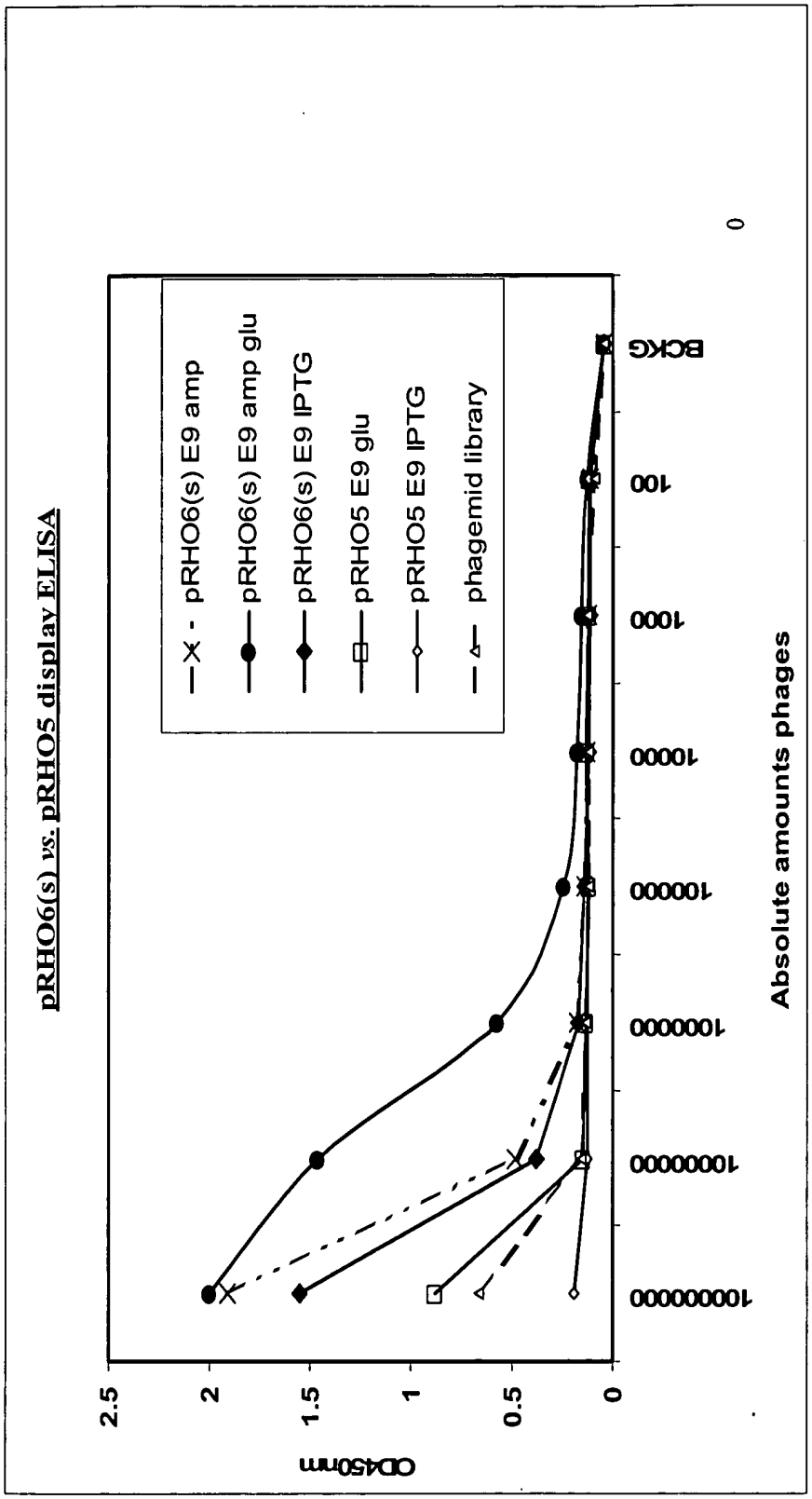
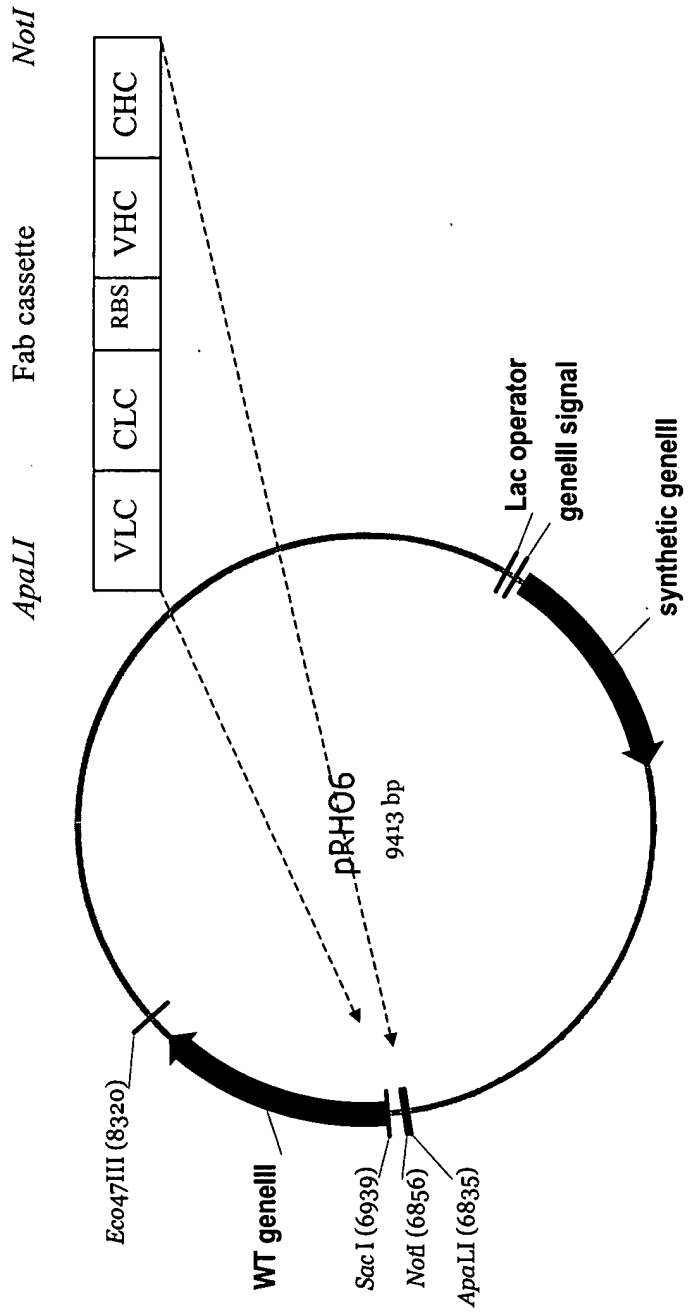

pRHO6(s) vs. pRHO5 display ELISA
OD450nm
2.5
2
1.5
1
0.5
0
Absolute amounts phages
BCKG
100
1000
10000
100000
1000000
10000000
100000000
- pRHO6(s) E9 amp
pRHO6(s) E9 amp glu
pRHO6(s) E9 IPTG
pRHO5 E9 glu
pRHO5 E9 IPTG
phagemid library

FIG. 5

FIG. 6


ApaLI
Fab cassette
NotI
VLC
CLC
RBS
VHC
CHC
pRH06
9413 bp
Lac operator
geneIII signal
synthetic geneIII
Eco47III (8320)
WT geneIII
Sac I (6939)
NotI (6856)
ApaLI (6835)

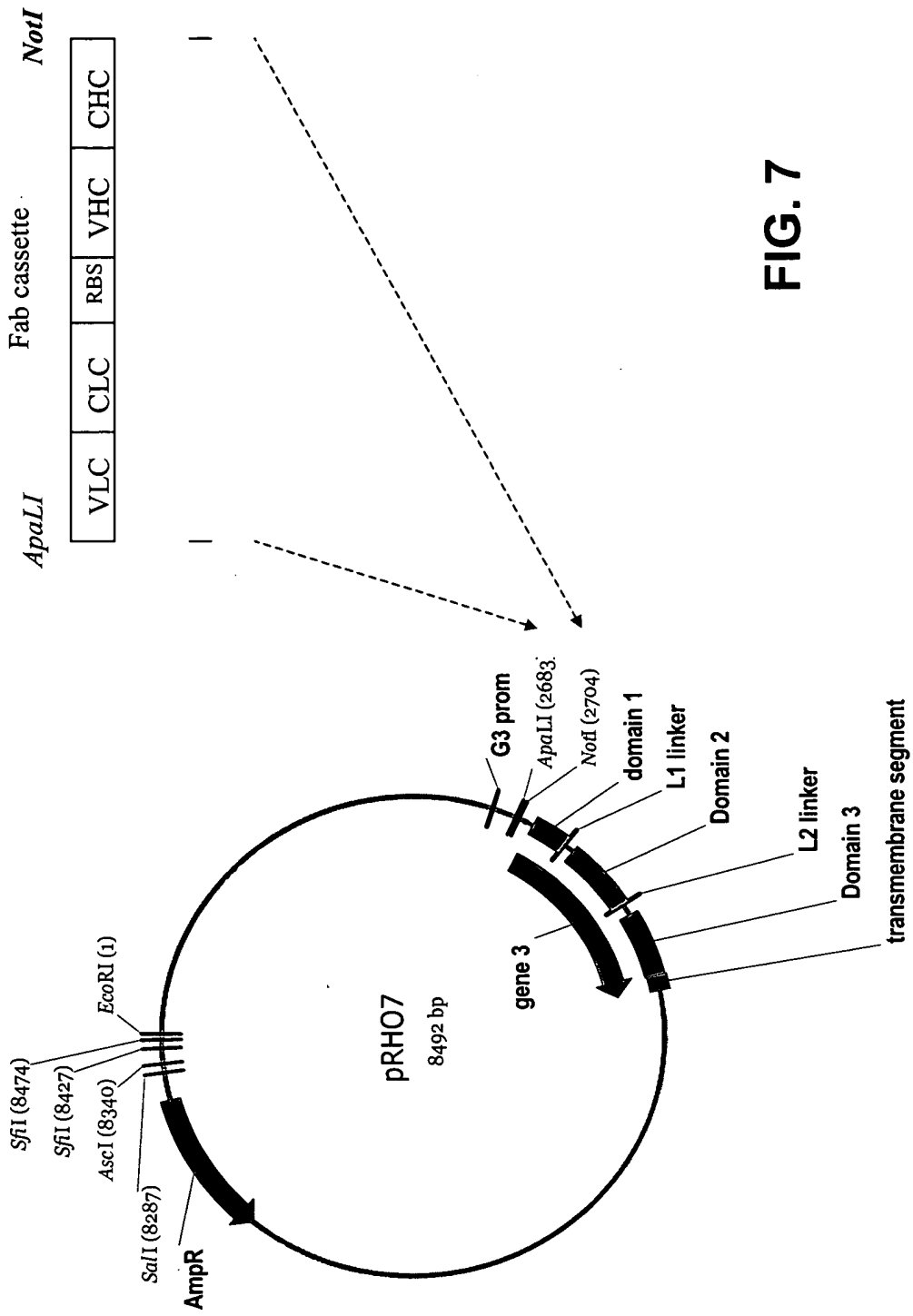

ApaLI
Fab cassette
NotI
VLC
CLC
RBS
VHC
CHC
pRHO7
8492 bp
G3 prom
ApaLI (2683)
NotI (2704)
domain 1
L1 linker
Domain 2
L2 linker
Domain 3
transmembrane segment
gene 3
EcoRI (1)
SfiI (8474)
SfiI (8427)
AscI (8340)
SalI (8287)
AmpR

FIG. 7

```
                                  → domain1
M13MP18 - wild type          1    TVESCLAKSH TENSFTNVWK DDKTLDRYAN YEGCLWNATG VVVCTGDETQ
PRH04+pRH06-S synthetic      1    TVESCLAKSH TENSFTNVWK DDKTLDRYAN YEGCLWNATG VVVCTGDETQ

                                       ←→ linker 1       ←→ domain 2
M13MP18 - wild type         51    CYGTWVPIGL AIPENEGGGS EGGGSEGGGS EGGGTKPPEY GDTPIPGYTY
PRH04+pRH06-S synthetic     51    CYGTWVPIGL AIPENEGGGS EGGGSEGGGS EGGGTKPPEY GDTPIPGYTY

M13MP18 - wild type        101    INPLDGTYPP GTEQNPANPN PSLEESQPLN TFMFQNNRFR NRQGALTVYT
PRH04+pRH06-S synthetic    101    INPLDGTYPP GTEQNPANPN PSLEESQPLN TFMFQNNRFR NRQGALTVYT

M13MP18 - wild type         51    GTVTQGTDPV KTYYQYTPVS SKAMYDAYWN GKFRDCAFHS GFNEDLFVCE
PRH04+pRH06-S synthetic    151    GTVTQGTDPV KTYYQYTPVS SKAMYDAYWN GKFRDCAFHS GFNEDPFVCE
                                                                                *

                                            ←→ linker 2
M13MP18 - wild type        201    YQGQSSDLPQ PPVNAGGGSG GGSGGGSEGG GSEGGGSEGG GSEGGGSGGG
PRH04+pRH06-S synthetic    201    YQGQSSDLPQ PPVNAGGGSG GGSGGGSEGG GSEGGGSEGG GSEGGGSGGG

                                  ←→ domain 3
M13MP18 - wild type        251    SGSGDFDYEK MANANKGAMT ENADENALQS DAKGKLDSVA TDYGAAIDGF
PRH04+pRH06-S synthetic    251    SGSGDFDYEK MANANKGAMT ENADENALQS DAKGKLDSVA TDYGAAIDGF
```

FIG. 8A

```
M13MP18 - wild type       301  IGDVSGLANG NGATGDFAGS NSQMAQVGDG DNSPLMNNFR QYLPSLPQSV
PRH04+pRH06-S synthetic   301  IGDVSGLANG NGATGDFAGS NSQMAQVGDG DNSPLMNNFR QYLPSLPQSV

                                                ←→ transmembrane segment←→
M13MP18 - wild type       351  ECRPFVFGAG KPYEFSIDCD KINLFRGVFA FLLYVATFMY VFSTFANILR
PRH04+pRH06-S synthetic   351  ECRPFVFSAG KPYEFSIDCD KINLFRGVFA FLLYVATFMY VFSTFANILR
                                       *

                               anchor
M13MP18 - wild type       401  NKES
PRH04+pRH06-S synthetic   401  NKES
```

FIG. 8B